Discovery of smallpox therapeutics that target processive DNA synthesis

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Smallpox (SP) Facts:

SP has killed hundreds of millions.

SP is caused by the virus, *Variola.*

Smallpox (SP) Facts:

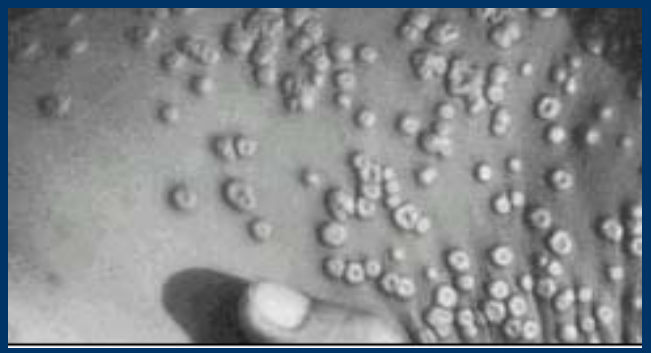
- SP was globally eradicated by vaccination in 1980.
- SP declared stocks exist in the USA and Russia.
- SP undeclared stocks pose a health threat.

Time Course of SP Infection:

Asymptomatic viremia. Only time that administration of vaccine will be effective.

Rash, fever , infectious spread saliva and clothing

Days post infection



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Our goal is to discover and develop therapeutics

that target the

Polymerase/Processivity complex of

vaccinia virus

The therapeutics are intended to:

Directly block *Variola* virus in rapid response to smallpox attack.

Protect individuals for whom the vaccine is contraindicated.

Processivity Factors

- Stabilize DNA polymerases on the template to enable extended strand synthesis
- Essential for viral replication
- Specific for their own polymerase

Identifying vaccinia proteins required for processive DNA synthesis

Vaccinia Processive DNA synthesis

DNA polymerase E9 incorporates ten nucleotides before dissociating from the template.

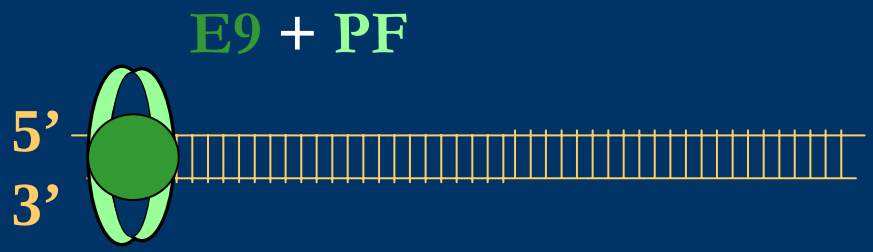
E9 alone is nonprocessive: short chains are synthesized

Nucleotide Incorporation of E9 alone

1 – Negative control
2 – Negative control
3 – E9

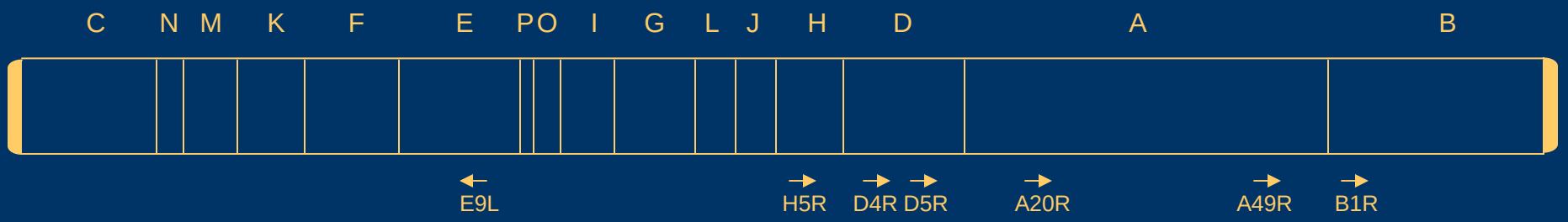
Vaccinia Processive DNA synthesis

Together with its processivity factor,
E9 incorporates thousands of nucleotides.



This extended strand synthesis is called
processive DNA synthesis, or processivity.

Discovering vaccinia proteins required for processive DNA synthesis



E9 replicative DNA polymerase

A20 putative processivity factor

D4 uracil DNA glycosylase

D5 DNA-independent dNTPase

B1 serine/threonine protein kinase

H5 substrate of B1 present in the virosomes

A49 protein that interacts with H5

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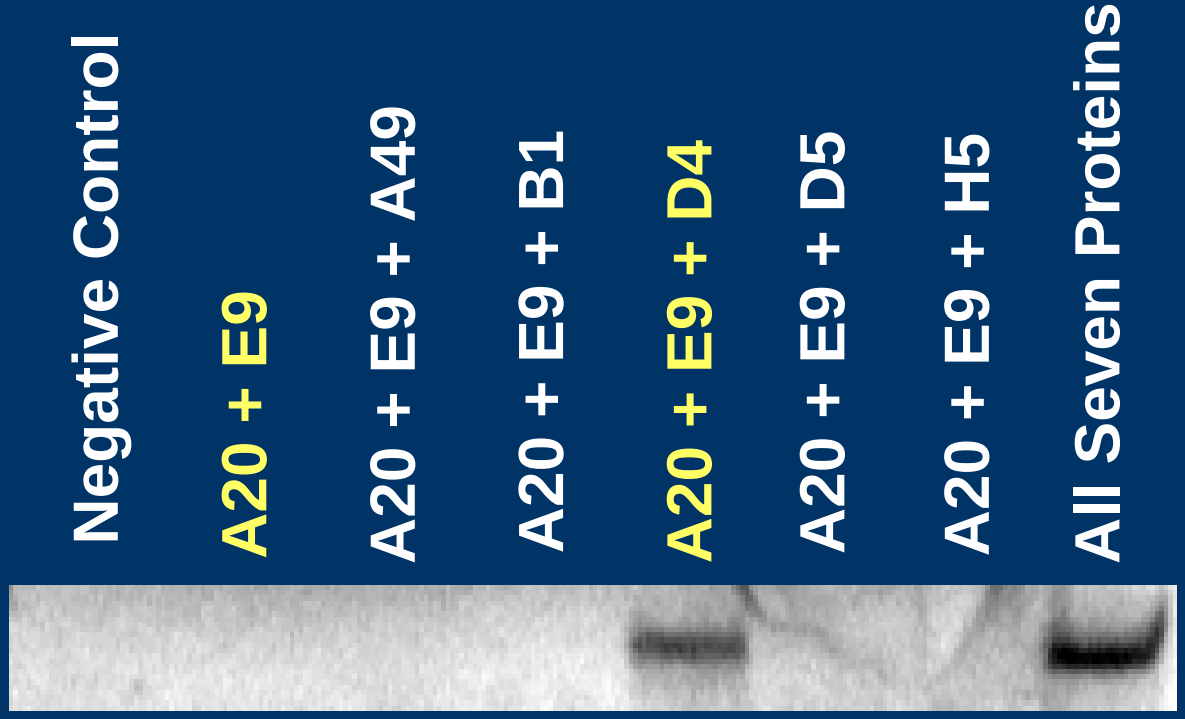
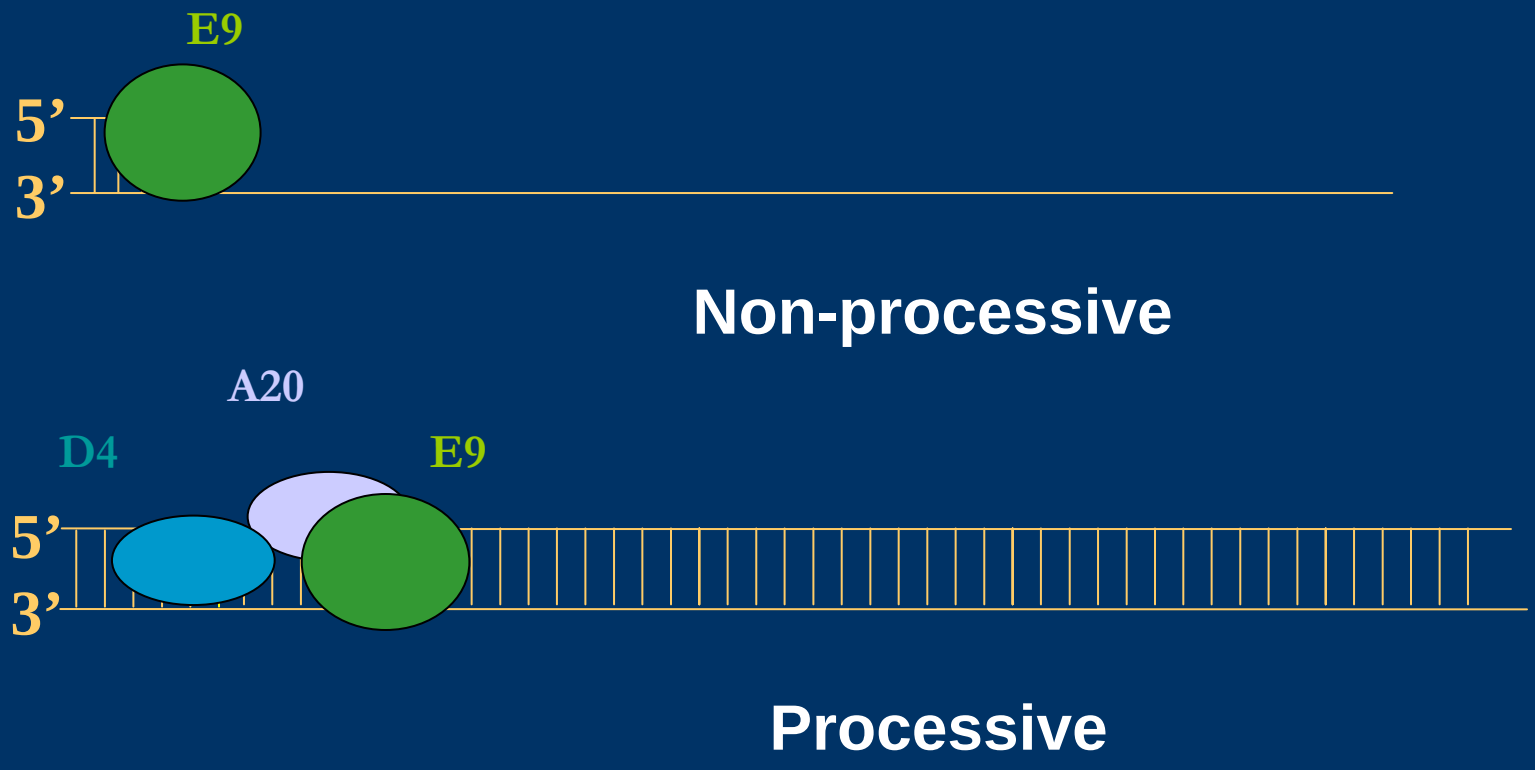
E9, A20 and D4 are required for processive DNA synthesis



A20, D4, and E9 are essential members of the vaccinia processivity complex



The vaccinia proteins A20, D4 and E9 share high sequence identity to their corresponding proteins in variola …

A20 identity = 98%

```
Vaccinia   MTSSADLTNLKELLSLYKSLKFSDSAAIEKYNSLVEWGTSTYWKIGVQKVANVETSISDY
Variola    MTSSADLTNLKELLSLYKSLRFSDSAAIEKYNSLVEWGTSTYWKIGVQKVANVETSISDY

Vaccinia   YDEVKNKPFNIDPGYYIFLPVYFGSVFIYSKGKNMVELGSGNSFQIPDDMRSACNKVLDS
Variola    YDEVKNKPFNIDPGYYIFLPVYFGSVFIYSKGKNMVELGSGNSFQIPDDMRSVCNKVLDG

Vaccinia   DNGIDFLRFVLLNNRWIMEDAISKYQSPVNIFKLASEYGLNIPKYLEIEIEEDTLFDDEL
Variola    DNGIDFLRFVLLNNRWIMEDAISKYQSPVNIFKLASEYGLNIPNYLEIEIEEDTLFDDEL

Vaccinia   YSIIERSFDDKFPKISISYIKLGELRRQVVDFFKFSFMYIESIKVDRIGDNIFIPSVITKSGKKI
Variola    YSIIERSFDDNFPKISISYIKLGELRRQVVDFFKFSFMYIESIKVDRIGDNIFIPSVITKSGKKI

Vaccinia   LVKDVDHLIRSKVREHTFVKVKKKNTFSILYDYDGNGTETRGEVIKRIIDTIGRDYYVNGKY
Variola    LVKDVDHLIRSKVREHTFVKVKKKNTFSILYDYDGNGTETRGEVIKRIIDTIGRDYYVNGKY

Vaccinia   FSKVGSAGLKQLTNKLDINECATVDELVDEINKSGTVKRKIKNQSAFDLSRECLGYPEA
Variola    FSKVGSAGLKQLTNKLNINECTTVDELVDEINKSGTVKRKIKTQSAFDLSRECLGYPEA

Vaccinia   DFITLVNNMRFKIENCKVVNFNIENTNCLNNPSIETIYGNFNQFVSIFNIVTDV KKRLFE
Variola    DFITLVNNMRFKIENCKVVNFNIENTNCLNNPSIETIYGNFNQFVSIFNIVTDV KKRLFE
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D4 identity = 97%

Vaccinia MNSVTVSHAPYTITYHDDWEPVMSQLVEFYNEVASWLLRDETSPIPDKFFIQLKQPLRNK

Variola MNSVTVSHAPYTITYHDDWEPVMNQLVEFYNEVASWLLRDETSPIPDKFFIQLKQPLRNK

Vaccinia RVCVCGIDPYPKDGTGVPFESPNFTKKSIKEIASSISRLTGVIDYKGYNLNIIDGVIPWN

Variola RVCVCGIDPYPKDGTGVPFESPNFTKKSIKEIASSISRLTGVIDYKGYNLNIIDGVIPWN

Vaccinia YYLSCKLGETKSHAIYWDKISKLLLQHITKHVSVLYCLGKTDYSNIRAKLESPVTTIVGY

Variola YYLSCKLGETKSHAIYWDKISKLLLHHITKHVRFLYCLGKTDFSNIRAKLESPVTTIVGY

Vaccinia HPAARDRQFEKDRSFEIINVLLELDNKAPINWAQGFIY

Variola HPAARDRQFEKDRSFEIINVLLELDNKAPINWAQGFIY

E9 identity = 98%

```
Vaccinia  MDVRCINWFESHGENRFLYLKSRCRNGETVFIRFPHYFYYVVTDEIYQSLSPPPFNARPLGKMRTIDIDETI
Variola   MDVRCINWFESHGENRFLYLKSRCRNGETVFIRFPHYFYYVVTDEIYQSLAPPPFNARPMGKMRTIDIDETI

Vaccinia  SYNLDIKDRKCSVADMWLIEEPKKRSIQNATMDEFLNISWFYISNGISPDGCYSLDEQYLTKINNGCYHCDD
Variola   SYNLDIKDRKCSVADMWLIEEPKKRNIQNATMDEFLNISWFYISNGISPDGCYSLDDQYLTKINNGCYHCGD

Vaccinia  PRNCFAKKIPRFDIPRSYLFLDIECHFDKKFPSVFINPISHTSYCYIDLSGKRLLFTLINEEMLTEQEIQEAVDR
Variola   PRNCFAKEIPRFDIPRSYLFLDIECHFDKKFPSVFINPISHTSYCYIDLSGKRLLFTLINEEMLTEQEIQEAVDR

Vaccinia  GCLRIQSLMEMDYERELVLCSEIVLLRIAKQLLELTFDYVVTFNGHNFDLRYITNRLELLTGEKIIFRSPDKKE
Variola   GCLRIQSLMEMDYERELVLCSEIVLLQIAKQLLELTFDYIVTFNGHNFDLRYITNRLELLTGEKIIFRSPDKKE

Vaccinia  AVHLCIYERNQSSHKGVGGMANTTFHVNNNNGTIFFDLYSFIQKSEKLDSYKLDSISKNAFSCMGKVLNRG
Variola   AVHLCIYERNQSSHKGVGGMANTTFHVNNNNGTIFFDLYSFIQKSEKLDSYKLDSISKNAFSCMGKVLNRG

Vaccinia  VREMTFIGDDTTDAKGKAAAFAKVLTTGNYVTVDEDIICKVIRKDIWENGFKVVLLCPTLPNDTYKLSFGKD
Variola   VREMTFIGDDTTDAKGKAAVFAKVLTTGNYVTVDD-IICKVIHKDIWENGFKVVLSCPTLTNDTYKLSFGKD

Vaccinia  DVDLAQMYKDYNLNIALDMARYCIHDACLCQYLWEYYGVETKTDAGASTYVLPQSMVFEYRASTVIKGPL
Variola   DVDLAQMYKDYNLNIALDMARYCIHDACLCQYLWEYYGVETKTDAGASTYVLPQSMVFEYKASTVIKGPL

Vaccinia  LKLLLETKTILVRSETKQKFPYEGGKVFAPKQKMFSNNVLIFDYNSLYPNVCIFGNLSPETLVGVVVSTNRL
Variola   LKLLLETKTILVRSETKQKFPYEGGKVFAPKQKMFSNNVLIFDYNSLYPNVCIFGNLSPETLVGVVVSSNRL

Vaccinia  EEEINNQLLLQKYPPPRYIVHCEPRLPNLISEIAIFDRSIEGTIPRLLRTFLAERARYKKMLKQATSSTEKTAI
Variola   EEEINNQLLLQKYPPPRYITVHCEPRLPNLISEIAIFDRSIEGTIPRLLRTFLAERARYKKMLKQATSSTEKAI

Vaccinia  YDSMQYTYKIVANSVYGLMGFRNSALYSYASAKSCTSIGRRMILYLESVLNGAELSNGMLRFANPLSNPFY
Variola   YDSMQYTYKIIANSVYGLMGFRNSALYSYASAKSCTSIGRRMILYLESVLNGAELSNGMLRFANPLSNPFY

Vaccinia  MDDRDINPIVKTSLPIDYRFRFRSVYGDTDSVFTEIDSQDVDKSIEIAKELERLINNRVLFNNFKIEFEAVYK
Variola   MDDRDINPIVKTSLPIDYRFRFRSVYGDTDSVFTEIDSQDVDKSIEIAKELERLINSRVLFNNFKIEFEAVYK

Vaccinia  NLIMQSKKKYTTMKYSASSNSKSVPERINKGTSETRRDVSKFHKNMIKTYKTRLSEMLSEGRMNSNQVCID
Variola   NLIMQSKKKYTTMKYSASSNSKSVPERINKGTSETRRDVSKFHKNMIKIYKTRLSEMLSEGRMNSNQVCID

Vaccinia  ILRSLETDLRSEFDSRSSPLELFMLSRMHHSNYKSADNPNMYLVTEYNKNNPETIELGERYYFAYICP
Variola   ILRSLETDLRSEFDSRSSPLELFMLSRMHHLNYKSADNPNMYLVTEYNKNNPETIELGERYYFAYICP

Vaccinia  ANVPWTKKLVNIKTYETIIDRSFKLGSDQRIFYEVYFKRLTSEIVNLLDNKVLCISFFERMFGSKPTFYEA
Variola   ANVPWTKKLVNIKTYETIIDRSFKLGSDQRIFYEVYFKRLTSEIVNLLDNKVLCISFFERMFGSRPTFYEA
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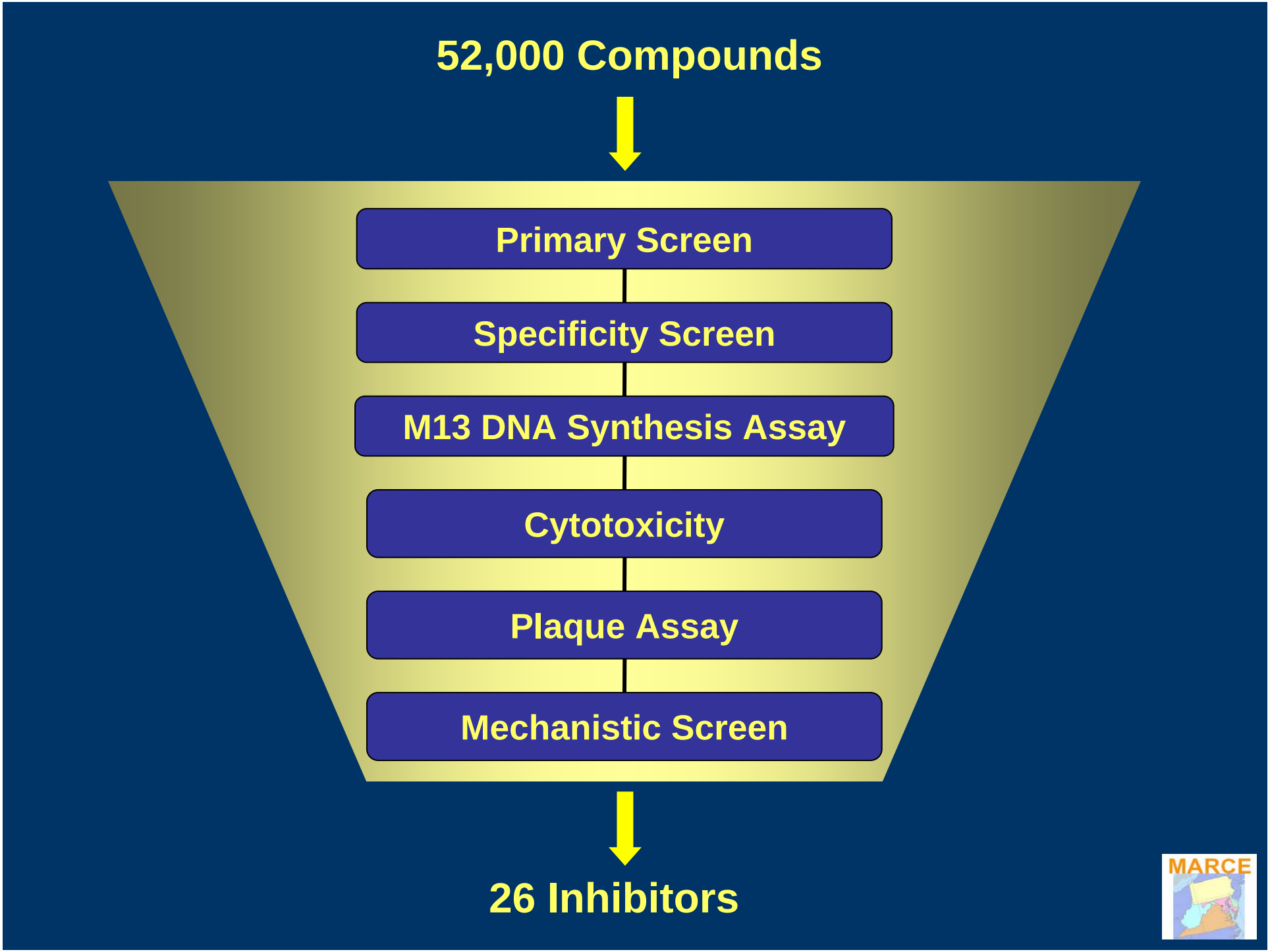
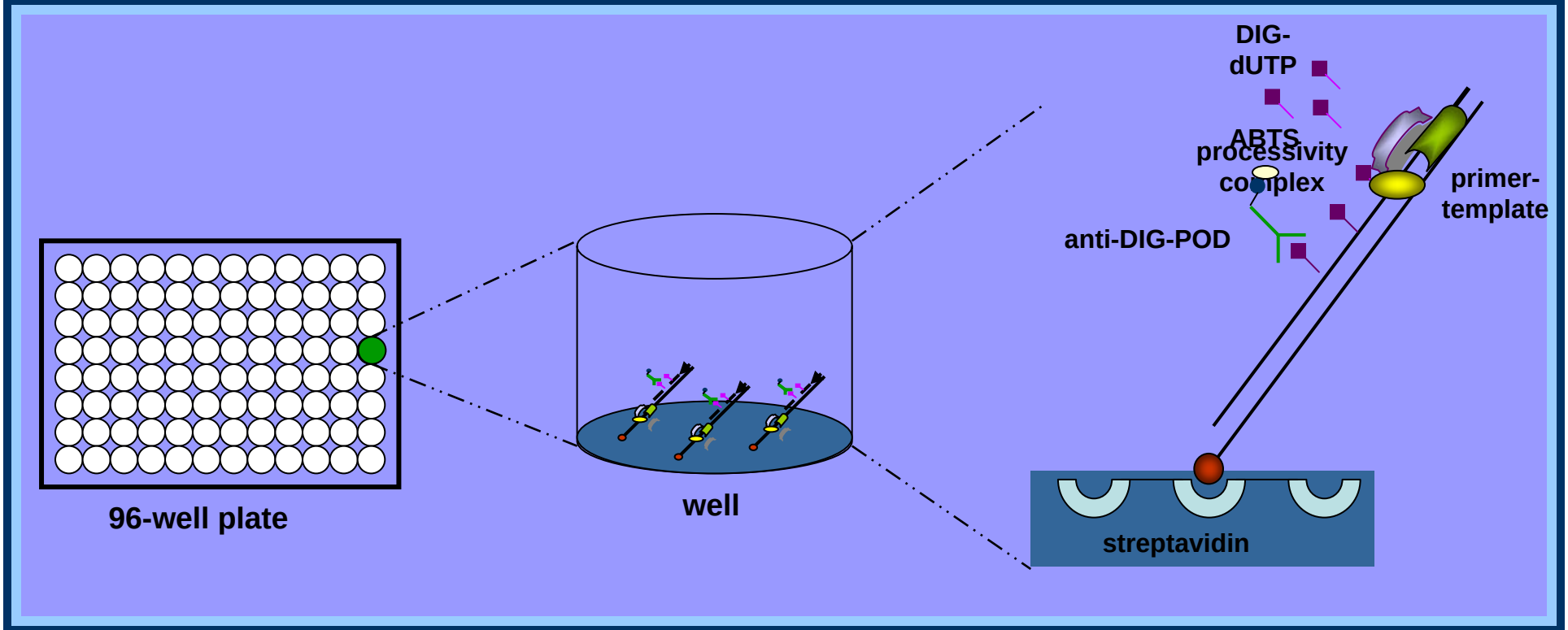
… which makes them excellent targets for

therapeutics against smallpox.

High-throughput screening of 52,000 compounds to identify inhibitors

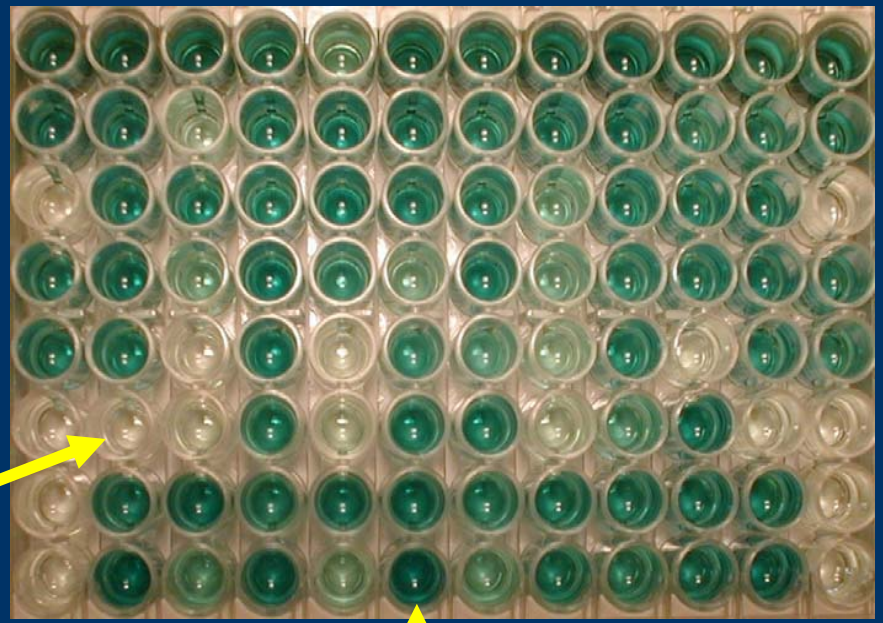

52,000 Compounds
Primary Screen
Specificity Screen
M13 DNA Synthesis Assay
Cytotoxicity
Plaque Assay
Mechanistic Screen
26 Inhibitors
MARCE

The Rapid Plate Assay



Lin, K., Ricciardi, R. P. *J. Virol. Methods.* (2000), 88, 219-225.
Dorjsuren, D., et al. Antiviral Research (2006), 69(1), 9-23.

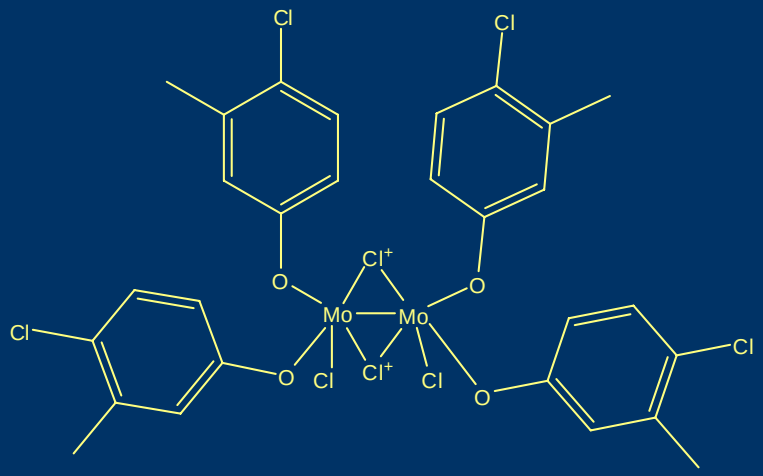
Rapid Assay Screening Plate



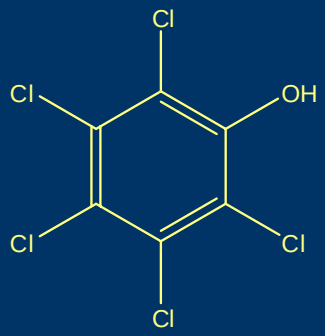
Inhibitor

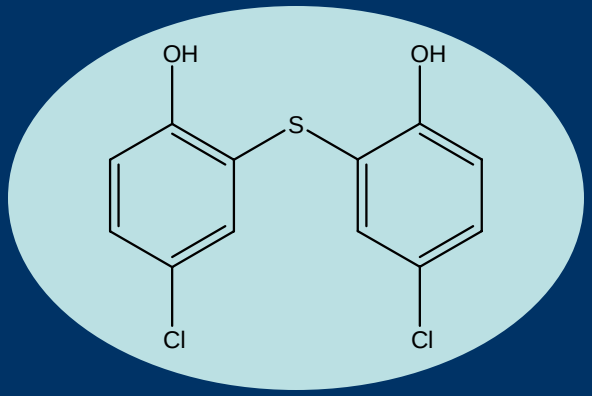
Non-inhibitor

Select Structural Analogs

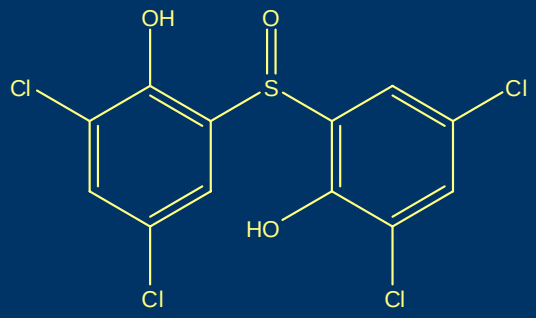


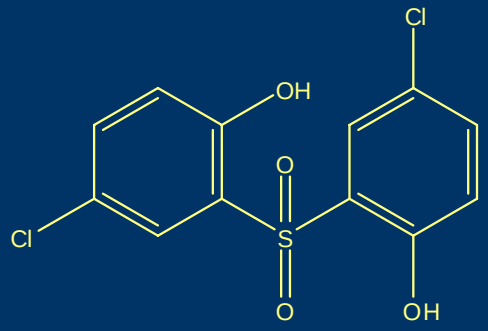
R

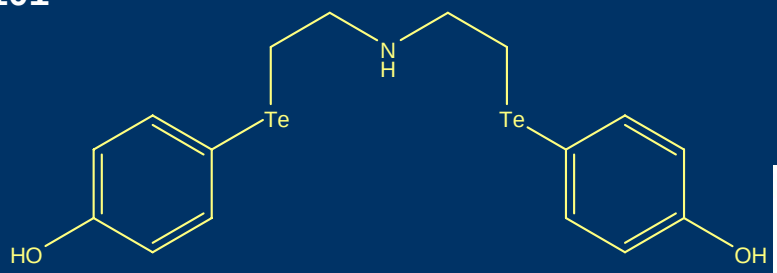




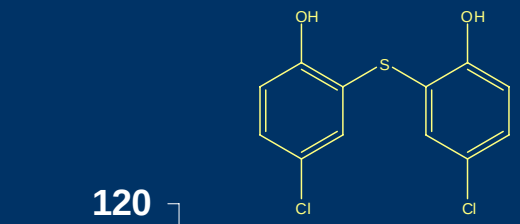
Parent M101

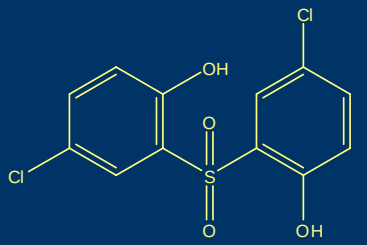


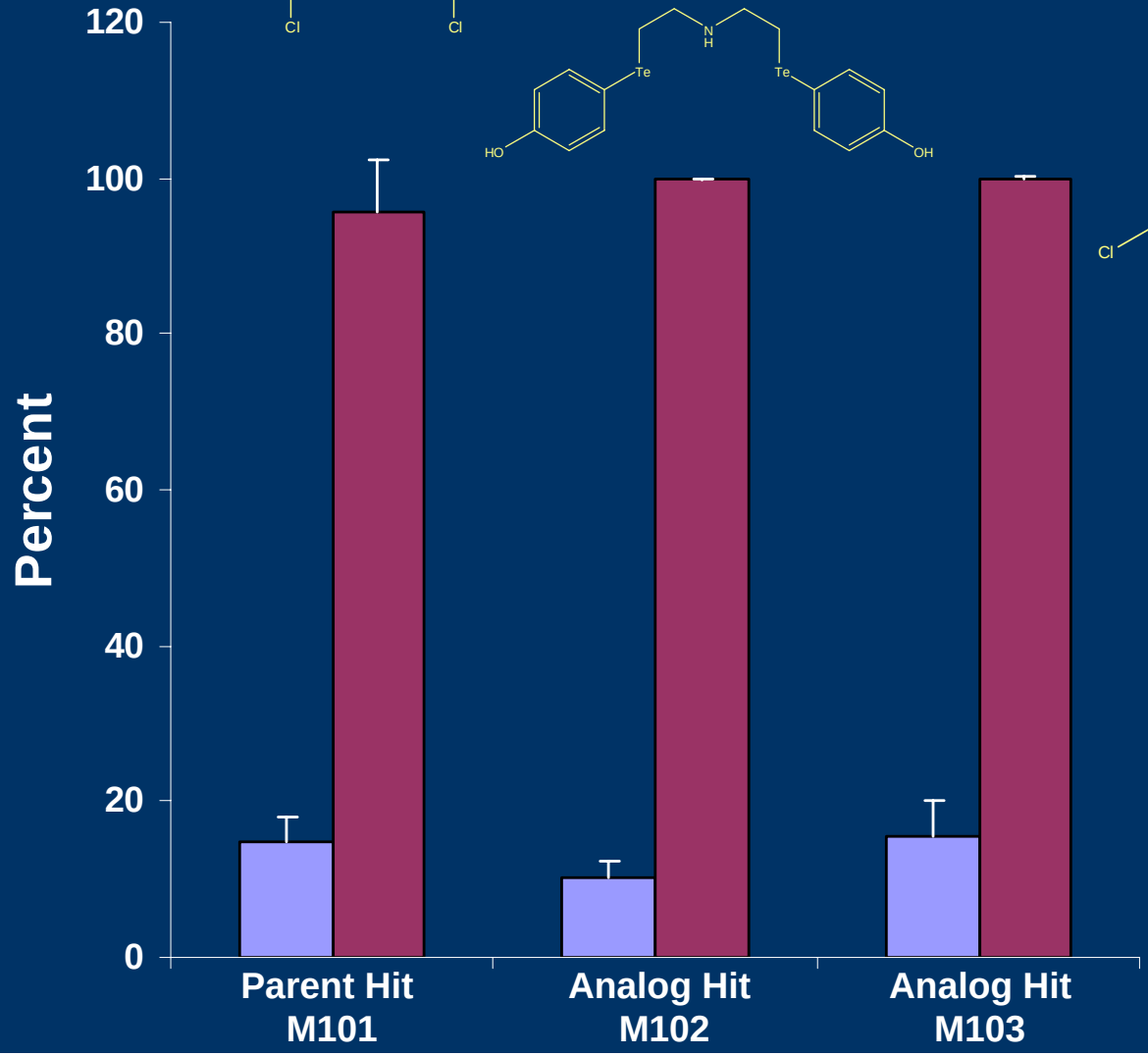




Viral plaques vs. cell toxicity







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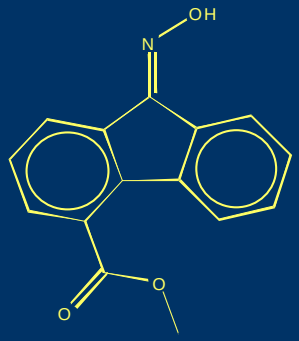
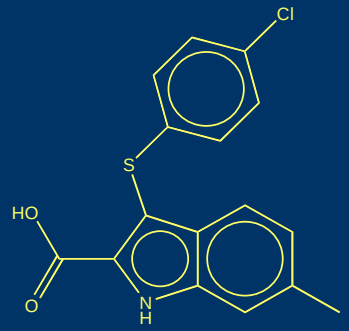
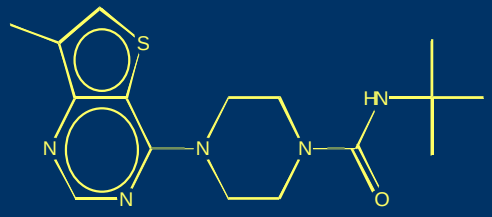
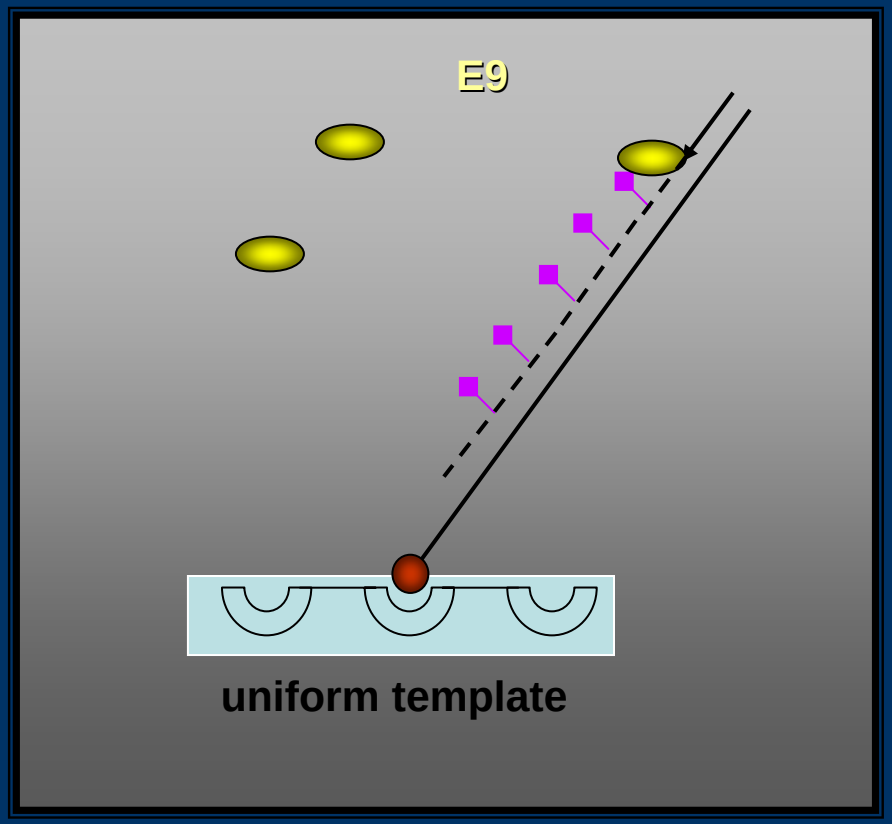
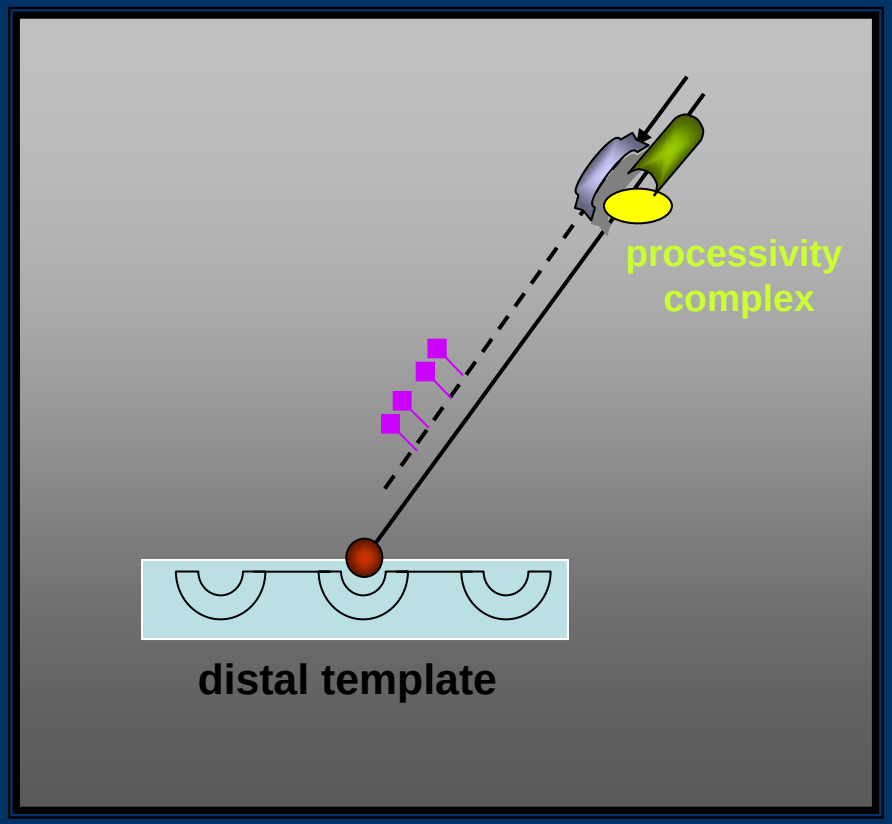
Inhibitors	*Structure*	*Plaque IC_{50}*	*Cytotoxicity IC_{50}*	*Therapeutic Index*
Cidofovir		**200 uM**	**50 uM**	**4**
M104		**127 uM**	**10 uM**	**12.7**
M105		**> 200 uM**	**75 uM**	**> 2.5**
M106		**159 uM**	**15 uM**	**10.6**

Mechanistic Assays

Catalytic

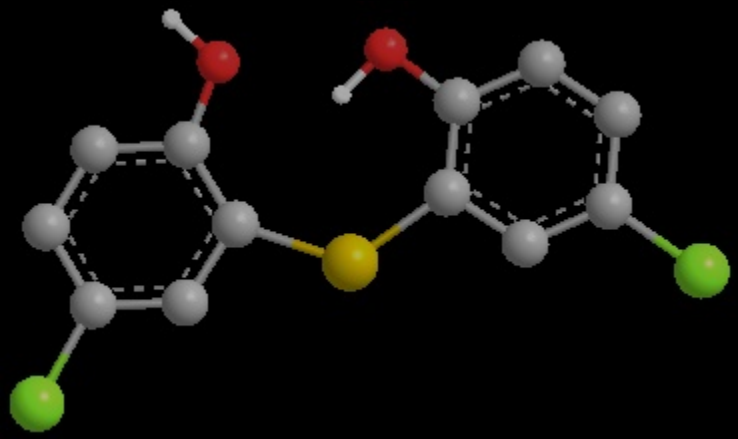


Processive



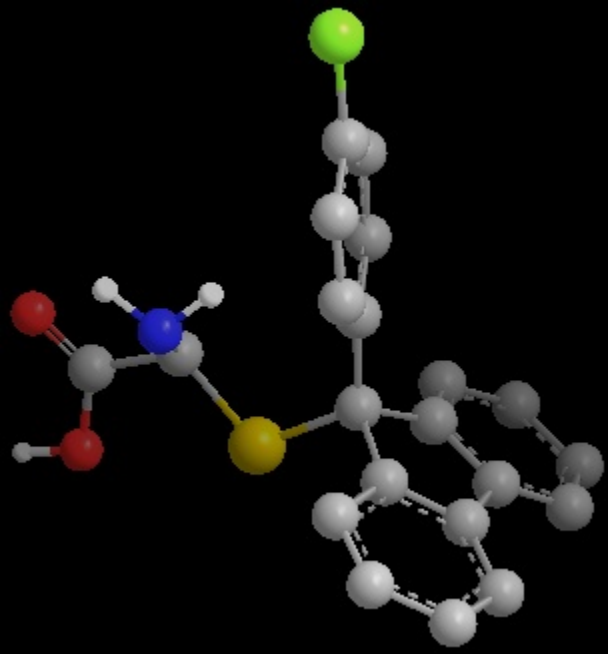
Mechanistic Assays

Catalytic



Mc 101

Processive



Mp 107

SUMMARY:

- **Discovered processivity proteins of vaccinia virus as therapeutic targets.**
- **Tested 52,000 chemicals for blocking DNA synthesis by HTS-Rapid Plate Assay.**
- **Identified 26 hit compounds that reduce infection with minimum cytotoxicity**

ONGOING STUDIES to OPTIMIZE the HITS:

- **Embarking on medicinal chemistry approach (Su Chiang group,** NSRB, Harvard).
- **Determine whether inhibitors disrupt A20, D4 and E9 interactions.**
- **Validate steps of vaccinia infection that are blocked by hits.**
- **Determine cellular uptake and half-life of inhibitors.**
- **Determine how inhibitors alter cell gene expression using microarray.**

Acknowledgements

- **NIH - MARCE FUNDING**
- **NCI Library *2,000 compounds* - Dr. Robert Shoemaker**
- **The National Screening Laboratory, Center of Excellence for Biodefense at Harvard Medical School, *50,000 compounds*- (Dr. Su Chiang, Assist Director Screening)**

Abigail M. Druck Shudofsky - *Identifying Proteins*
Janice Elaine P. Young - *Assay Design and Small Screen*
Mihai Ciustea - *High-Throughput Screening*